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# MODEL-BASED OPTIMIZATION TO EXPLAIN LIVER ZONATION IN NITROGEN METABOLISM

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## ABSTRACT

Model-based optimization (MBO) is used to explain the zonation of nitrogen metabolism in the mammalian liver based on an established model [1, 2]. The model has two compartments, one describing the periportal (pp) and one the pericentral (pc) zone of the liver lobule acinus. It is based on liver perfusion data [3] and describes for the pp zone glutamine breakdown to ammonia (enzyme: glutaminase) and ammonia detoxification to urea (key enzyme: carbamoyl phosphate synthetase) and for the pc zone glutamine synthesis (glutamine synthetase).

The MBO applied determines optimal enzyme activity distributions along the compartments using a nonlinear programming algorithm [4]. All reactions are allowed to take place in each compartment, i.e. there is no pp or pc zone assumed *a priori*. The optimization problem is formulated based on the modified and extended original model, on biologically motivated enzyme constraints and objective functions that represent different physiological strategies of the liver.

The MBO is first based on the modified two-compartment model. Using objective functions that consider either only ammonia minimization or urea maximization do either not reflect the zonal structure or the *in vivo* enzyme distribution. The objective function is therefore reformulated to reflect besides ammonia minimization and urea maximization also glutamine maintenance. This results in an optimal enzyme activity distribution that reflects the original two-compartment structure well. Based on this, the MBO is then extended to consider 16 compartments (hepatocytes) that correspond to the average hepatocyte number from the pp to the pc site. This results in a narrow pc zone dedicated exclusively to glutamine synthesis, which is experimentally well supported [5]. For the large 'pp' zone, however, the MBO results suggest that this zone is further subdivided providing interesting explanations for unexpected experimental findings [6, 7].

***Index Terms*** – Liver zonation, nitrogen metabolism, optimal design, model-based optimization in biology and medicine

## 1. INTRODUCTION

The liver is a highly structured organ composed of identical subunits, the liver lobuli. Ammonia detoxification and glutamine regulation are two major tasks of the liver lobuli. Each lobule consists of a pp and a pc zone. Modelling makes it possible to simulate the metabolic reactions in the liver lobule *in silico*.

An established model [1, 2], described in Section 2, with a two-compartment structure, one compartment for the pp and one for the pc zone, represents data from rat liver perfusion experiments [3] very well. It describes for the pp zone glutamine breakdown to ammonia by the enzyme glutaminase and ammonia detoxification to urea by the enzyme carbamoyl phosphate synthetase and for the pc zone glutamine synthesis by the enzyme glutamine synthetase.

Here, a new approach, described in Section 3, is proposed to identify the enzyme activities of the metabolic reactions in the liver lobule using optimization techniques, in particular a nonlinear programming algorithm. Optimization approaches have also been applied with respect to metabolic pathway analysis, e.g. steady-state analysis [8, 9] and dynamic analysis [10, 11]. For the MBO, all reactions are allowed to take place in each compartment, i.e. there is no pp or pc zone assumed *a priori*. The aim is to determine optimal enzyme activity distributions along the compartments. Based on the original model, biologically motivated objective functions (representing different physiological strategies of the liver) are formulated and constraints on the enzymes introduced to define and solve the optimization problem.

The results of this MBO approach are presented in Section 4. The optimization was initially based on the two-compartment model and an objective function that solely considers ammonia detoxification (minimization). This results only in activities of the enzymes for ammonia detoxification, i.e. using this function does not reflect the zonal structure. Then an objective function that solely considers urea formation (maximization) was used. The results reflect the zonal structure but the obtained enzyme distribution is not in line with experimental observa-

tions. Since however ammonia detoxification to urea as well as maintenance of glutamine are major tasks of the liver, the objective function was reformulated to reflect besides ammonia minimization and urea maximization also glutamine maintenance. The optimal enzyme activity distribution obtained using this objective function structurally reflects the pp and the pc zone of the original two-compartment model [1, 2]. Based on this objective function, the MBO was then extended to consider 16 compartments (hepatocytes) that correspond to the average number of hepatocytes from the pp to the pc site in the rat liver. The identified small pc zone resembles the pc zone found *in vivo* [5] very well. However, the enzyme activity distribution obtained for the large 'pc' zone indicates in agreement with other experimental findings [6, 7] that this zone may have to be further subdivided.

## 2. MODEL OF LIVER NITROGEN METABOLISM

The model used in this study for MBO was taken from [1, 2] and is based on data from rat liver perfusion experiments with input and output measurements [3]. It structurally takes into account two compartments, one for the pp and one for the pc zone (see Fig. 1). The model structure was established in [1, 2] using biological knowledge from [3, 5, 12-15]. The model considers for the pp zone glutamine breakdown to ammonia as well as ureogenesis, where glutamine breakdown is catalysed by the enzyme glutaminase $e_G$ and ureogenesis by carbamoyl phosphate synthetase $e_C$. For the pc zone, the model takes into account glutamine synthesis catalysed by glutamine synthetase $e_{GS}$. The release of endogenous ammonia ($c_{NH4,Endog1/2}$) as caused by the breakdown of endogenous proteins and amino acids mainly in the pp zone [1, 2] is also considered by the model.

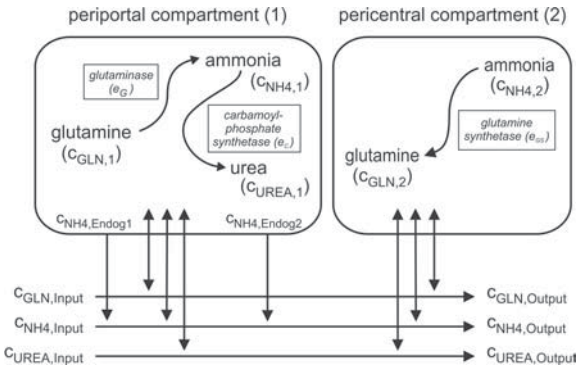


*Figure 1: Outline of the two-compartment model of liver nitrogen metabolism (modified from [15]) used for MBO.*

The concentrations of glutamine $c_{GLN}$, ammonia $c_{NH4}$ and urea $c_{UREA}$ are described by the following set of differential equations

for the pp compartment

$$\begin{aligned}
\dot{c}_{GLN,1} &= -\frac{1}{Y_G}\cdot v_G(c_{GLN,1}, c_{NH4,Input}) + \frac{F}{V_1}(c_{GLN,Input} - c_{GLN,1}) \\
\dot{c}_{NH4,1} &= v_G(c_{GLN,1}, c_{NH4,Input}) - \frac{1}{Y_C}\cdot v_C(c_{NH4,1}) \\
&\quad + \frac{F}{V_1}\cdot(c_{NH4,Input} + c_{NH4,Endog1} - c_{NH4,1}) \\
\dot{c}_{UREA,1} &= v_C(c_{NH4,1}) + \frac{F}{V_1}(c_{UREA,Input} - c_{UREA,1})
\end{aligned} \tag{1}$$

and for the pc compartment

$$\begin{aligned}
\dot{c}_{GLN,2} &= v_{GS}(c_{NH4,2}, c_{GLN,2}) + \frac{F}{V_2}(c_{GLN,1} - c_{GLN,2}) \\
\dot{c}_{NH4,2} &= -\frac{1}{Y_{GS}} v_{GS}(c_{NH4,2}, c_{GLN,2}) \\
&\quad + \frac{F}{V_2}\cdot(c_{NH4,1} + c_{NH4,Endog2} - c_{NH4,2}) \\
\dot{c}_{UREA,2} &= \frac{F}{V_2}(c_{UREA,1} - c_{UREA,2})
\end{aligned} \tag{2}$$

where the perfusion flow $F$ passes through the compartments and transports the metabolites from the input site of the pp zone to the output site of the pc zone. The two compartments have the volumes $V_1=15V_2$ and $V_2$, since the average number of hepatocytes from the pp to the pc site in the rat liver equals 16 of which 15 are assigned to the pp zone and only one to the pc zone. The differential equations describe the concentration changes caused by the enzyme catalysed reactions (by $e_G$, $e_C$ and $e_{GS}$) and by the perfusion stream $F$ dependent on the different compartment volumes. The metabolite concentrations of the pc compartment equal the output concentrations as shown in Fig. 1. The reaction rates $v_G$, $v_C$ and $v_{GS}$ are modelled by Michaelis-Menten terms [2].

This two-compartment model has altogether been established knowledge-based with respect to model structure determination and data-based with respect to model parameter estimation. It has also been shown in [2] that an one-compartment model is incapable of reproducing the experimental data from [3]. On the other hand, from the model identification point of view, the amount of experimental data available [3] would not allow to fit a much more complex model than the one with the two-compartment structure and the considered reactions. For the MBO however, as described in Section 3, the two-compartment model has been modified (see Subsection 3.1.) and additionally been extended to sixteen compartments that correspond to the average hepatocyte number from the pp to the pc site of the rat liver (see Subsection 3.2.) in order to investigate zonation along this number of hepatocytes.

## 3. MBO METHOD TO DETERMINE OPTIMAL ZONATION

A new approach is proposed here to determine, based on the model described in Section 2., the enzyme activities of the metabolic reactions in the liver lobule using optimization techniques, in particular the nonlinear programming algorithm SNOPT [4].

For the MBO, all three metabolic reactions of the original model (glutamine breakdown, ureogenesis and glutamine synthesis) are allowed to take place in each of the considered compartments, i.e. there is no pp or pc zone assumed *a priori*. The aim of the optimization is to determine optimal enzyme activity distributions along the compartments (hepatocytes) with respect to a biologically meaningful objective function under enzyme activity constraints (see Section 4). Several case studies to formulate an adequate objective function are also presented in Section 4.

The MBO is used to predict the model structure based on the solution of an optimization problem with the enzyme activities as independent or optimization variables. Predicting the model structure here means identifying a specific structure within a pre-defined knowledge-based potential structure (the modified two-compartment and the extended sixteen-compartment model, see Subsections 3.1. and 3.2.) by optimizing parameters (the enzyme activities) that determine this structure. If the predictions are in line with actual observations, one can say that the identified structure is adapted to the task formulated by the optimization problem. The optimization problem is defined in detail in Section 4.

### 3.1. Modified two-compartment model

The MBO approach outlined so far is based on the two-compartment model given in Section 2. There are however some model modifications necessary to allow all metabolic reactions to take place in each compartment.

The MBO determines the model structure based on the identification of enzyme activity distributions. Here, the relative enzyme activations with respect to the experimental conditions in [3] are used. The relative enzyme activations can be described with the respect to the MBO by simply multiplying the reaction rates $v_G$, $v_C$ and $v_{GS}$ with the corresponding enzyme activities $e_{G,j}$, $e_{C,j}$ and $e_{GS,j}$ ($j=1, 2$). Additionally, in this work time-independent concentrations are considered (the time-derivatives are equal to zero). The modified model equations derived from Eq. (1) are now for the pp compartment

$$0 = \mathbf{e_{GS,1}} \cdot \mathbf{v_{GS}}(\mathbf{c_{NH4,1}}, \mathbf{c_{GLN,1}}) - \frac{1}{\mathbf{Y_G}} \cdot \mathbf{e_{G,1}} \cdot \mathrm{v_G}(c_{GLN,1}, c_{NH4,Input}) + \frac{F}{V_1}(c_{GLN,Input} - c_{GLN,1}) \tag{3}$$

$$0 = -\frac{1}{\mathbf{Y_{GS}}} \cdot \mathbf{e_{GS,1}} \cdot \mathbf{v_{GS}}(\mathbf{c_{NH4,1}}, \mathbf{c_{GLN,1}}) + \mathbf{e_{G,1}} \cdot \mathrm{v_G}(c_{GLN,1}, c_{NH4,Input}) - \frac{1}{Y_C} \cdot \mathbf{e_{C,1}} \cdot \mathrm{v_C}(c_{NH4,1}) + \frac{F}{V_1} \cdot (c_{NH4,Input} + c_{NH4,Endog1} - c_{NH4,1})$$

$$0 = \mathbf{e_{C,1}} \cdot \mathrm{v_C}(c_{NH4,1}) + \frac{F}{V_1}(c_{UREA,Input} - c_{UREA,1}).$$

where the extensions of the reaction rates and enzyme activities are highlighted in bold. Similarly, the modified model equations can be derived from Eq. (2) for the pc compartment

(4)

$$0 = \mathbf{e_{GS,2}} \cdot \mathrm{v_{GS}}(c_{NH4,2}, c_{GLN,2}) - \frac{1}{\mathbf{Y_G}} \cdot \mathbf{e_{G,2}} \cdot \mathbf{v_G}(\mathbf{c_{GLN,2}}, \mathbf{c_{NH4,1}}) + \frac{F}{V_2}(c_{GLN,1} - c_{GLN,2})$$

$$0 = -\frac{1}{Y_{GS}} \cdot \mathbf{e_{GS,2}} \cdot \mathrm{v_{GS}}(c_{NH4,2}, c_{GLN,2}) + \mathbf{e_{G,2}} \cdot \mathbf{v_G}(\mathbf{c_{GLN,2}}, \mathbf{c_{NH4,1}}) - \frac{1}{\mathbf{Y_C}} \cdot \mathbf{e_{C,2}} \cdot \mathbf{v_C}(\mathbf{c_{NH4,2}}) + \frac{F}{V_2} \cdot (c_{NH4,1} + c_{NH4,Endog2} - c_{NH4,2})$$

$$0 = \mathbf{e_{C,2}} \cdot \mathbf{v_C}(\mathbf{c_{NH4,2}}) + \frac{F}{V_2}(c_{UREA,1} - c_{UREA,2}).$$

As for the model given in Section 2, the metabolite concentrations of the pc compartment are equal to the output concentrations as shown in Fig. 1.

The six nonlinear equations in Eqs. (3), (4) describe the steady-state dependencies of the input and the compartmental concentrations. The six enzyme activities of the compartments are optimized by the nonlinear programming algorithm.

### 3.2. Extended sixteen-compartment model

The MBO is also applied to a sixteen-compartment model. These sixteen compartments represent sixteen individual hepatocytes corresponding to the average hepatocyte number from the pp to the pc site in the rat liver.

For the extension from two to sixteen compartments the volume $V$ of each compartment can be calculated by

$$V = (V_1 + V_2)/16. \tag{5}$$

This means that the original pp zone of the two-compartment model is divided into fifteen compartments or hepatocytes.

The endogenous sources of ammonia are considered equally distributed over all compartments and can be interpreted as internal ammonia sources $c_{NH4,Endog}$ in the individual hepatocytes

$$c_{NH4,Endog} = (c_{NH4,Endog1} + c_{NH4,Endog2})/16. \tag{6}$$

For the MBO, again all metabolic reactions are allowed to take place in each of the now sixteen compartments and factors representing their enzyme activities are introduced.

With the above modifications, the nonlinear equations from Eq. (3) for the first compartment can be rewritten as (changes again highlighted in bold)

(7)

$$0 = e_{GS,1} \cdot v_{GS}(c_{NH4,1}, c_{GLN,1}) - \frac{1}{Y_G} \cdot e_{G,1} \cdot v_G(c_{GLN,1}, c_{NH4,Input}) + \frac{F}{V}(c_{GLN,Input} - c_{GLN,1})$$

$$0 = -\frac{1}{Y_{GS}} \cdot e_{GS,1} \cdot v_{GS}(c_{NH4,1}, c_{GLN,1}) + e_{G,1} \cdot v_G(c_{GLN,1}, c_{NH4,Input}) - \frac{1}{Y_C} \cdot e_{C,1} \cdot v_C(c_{NH4,1}) + \frac{F}{V} \cdot (c_{NH4,Input} + \mathbf{c_{NH4,Endog}} - c_{NH4,1})$$

$$0 = e_{C,1} \cdot v_C(c_{NH4,1}) + \frac{F}{V}(c_{UREA,Input} - c_{UREA,1}).$$

From Eq. (4), the nonlinear equations for the second to the sixteenth compartment can be derived as

(8)

$$0 = e_{GS,\mathbf{i}} \cdot v_{GS}(c_{NH4,\mathbf{i}}, c_{GLN,\mathbf{i}}) - \frac{1}{Y_G} \cdot e_{G,\mathbf{i}} \cdot v_G(c_{GLN,\mathbf{i}}, c_{NH4,\mathbf{i-1}}) + \frac{F}{V}(c_{GLN,\mathbf{i-1}} - c_{GLN,\mathbf{i}})$$

$$0 = -\frac{1}{Y_{GS}} \cdot e_{GS,\mathbf{i}} \cdot v_{GS}(c_{NH4,\mathbf{i}}, c_{GLN,\mathbf{i}}) + e_{G,\mathbf{i}} \cdot v_G(c_{GLN,\mathbf{i}}, c_{NH4,\mathbf{i-1}}) - \frac{1}{Y_C} \cdot e_{C,\mathbf{i}} \cdot v_C(c_{NH4,\mathbf{i}}) + \frac{F}{V} \cdot (c_{NH4,\mathbf{i-1}} + \mathbf{c_{NH4,Endog}} - c_{NH4,\mathbf{i}})$$

$$0 = e_{C,\mathbf{i}} \cdot v_C(c_{NH4,\mathbf{i}}) + \frac{F}{V}(c_{UREA,\mathbf{i-1}} - c_{UREA,\mathbf{i}}).$$

with *i=2,...,16*. Here, the metabolite concentrations of the sixteenth compartment equal the corresponding output concentrations as shown in Fig. 1.

Based on the altogether 48 nonlinear equations Eqs. (7), (8), the 48 enzyme activities of the 16 compartments (hepatocytes) are optimized by the nonlinear programming algorithm.

## 4. MBO RESULTS AND OPTIMAL ZONATION

The idea of the presented MBO is to determine enzyme activity distributions by solving an optimization problem with constraints.

The enzyme activities are constrained here relative to the experiments in [3] by

$$0 \le e_{GS,j}, e_{G,j}, e_{C,j} \le 1,\ j = 1, ..., n \tag{9}$$

where *n* represents the number of compartments.

But also, the aspect that a cell can only synthesize a certain amount of enzymes needs to be taken into account. This can be done by imposing additional constraints on the enzyme activities in each compartment

$$e_{GS,j} + e_{G,j} + e_{C,j} \le e_{max},\ j = 1, ..., n\ . \tag{10}$$

Several such constraints are discussed in [9, 10]. Using high $e_{max}$ values will lead to the inactivity of these constraints. That is why unnatural optimization results may be obtained for high $e_{max}$ values. Therefore $e_{max}$=1 is chosen here representing the minimum value for the model structure (cf. compartment 2 in Fig. 1 and Eqs. (2), (4), (10)). Using this relatively small value, a high activity of an enzyme as optimization result indicates a high profit with respect to the minimization of the objective function. If more than one enzyme activation is profitable, the available amount can be spread across several enzymes in the compartments.

In addition to this, further data is needed to solve the optimization problem. This refers to the input concentrations for glutamine, ammonia and urea which were also taken from [3]. Here, values from an experiment where conditions are closest to *in vivo* situations with the experimental input concentrations $c_{GLN,Input}=0.5mM$ for glutamine, $c_{NH4,Input}=0.3mM$ for ammonia and $c_{UREA,Input}=0mM$ for urea were choosen. The corresponding measured output concentrations are $c_{GLN,Output} \sim 0.45mM$ and $c_{UREA,Output} \sim 0.2mM$, whereas the ammonia concentration is not available.

The optimization studies described in the following subsections are based on Eqs. (3), (4) for the modified two-compartment model (Subsection 4.1.) and on Eqs. (7), (8) for the extended sixteen-compartment model (Subsection 4.2.).

### 4.1. MBO-based confirmation of the two-compartment model structure

This subsection describes how the objective function is formulated based on biological knowledge and the optimization results obtained based on the modified two-compartment model (see Subsection 3.1.).

The optimal enzyme distribution to be obtained by the MBO should reflect the original two-compartment model structure. If this is not the case, a reformulation of the objective function would be necessary. It should be noted again that a spread of the enzyme activations is allowed and can thus be an optimization result.

One of the main physiological tasks of the liver is the detoxification of ammonia from the bloodstream that finally leaves the output site (the pc zone). This can be formulated by the following objective function

$$\min_{\mathbf{e}}\ c_{NH4,2} \tag{11}$$

where $\boldsymbol{e}$ represents the vector of all enzyme activities

$$\mathbf{e} = [e_{GS,1}, e_{G,1}, e_{C,1}, e_{GS,2}, e_{G,2}, e_{C,2}]^T\ . \tag{12}$$

The resulting optimization problem is therefore formulated by the objective function Eq. (11), the equality constraints, i.e. the model Eqs. (3), (4), and the inequality constraints on the enzyme activities Eqs. (9), (10) with *n=2*.

The optimal enzyme activity distribution obtained using Eq. (11) as objective function (Fig. 2) shows only activity of glutamine synthetase in both zones, i.e. in the pp zone (first compartment) and in the pc zone (second compartment). This means there is a

high activity of this enzyme in both zones to detoxify the input concentration of ammonia. It can be seen that ammonia is completely removed, but no urea is formed and only glutamine is released. Using this objective function alone obviously does not reflect the original two-compartment structure, since the identical enzyme activations in both compartments actually reflect a one-compartment structure.

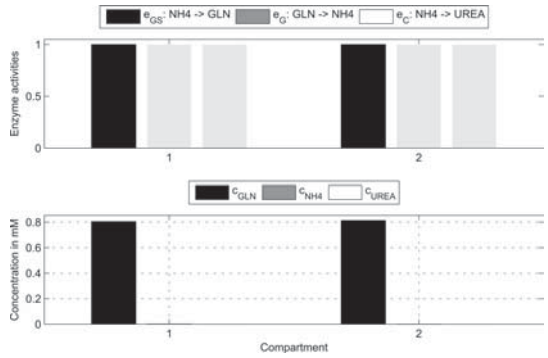


*Figure 2: Optimization results for the two-compartment model and for ammonia minimization only (Eq.(11)) with the obtained enzyme activity distribution (upper part) and the corresponding metabolite concentrations (lower part).*

However, it is well-known that physiologically the excretion of redundant nitrogen is realised via ureogenesis (and not glutamine formation). This can be formulated by the following objective function

$$\max_{e} \; c_{UREA,2} \,. \tag{13}$$

The optimization results obtained using Eq. (13) as objective function are shown in Fig. 3. The original compartmental model structure is now reflected. But although in the pp zone both glutaminase and carbamoyl phosphate synthetase are active, there is no activation of glutamine synthetase in the pc zone. There are also relatively high concentrations of ammonia released.

While Fig. 3 shows a compartmental enzyme activation and for the pp zone the activation of the original model, Fig. 2 shows no compartmental enzyme activation but for the pc zone the activation of the original model.

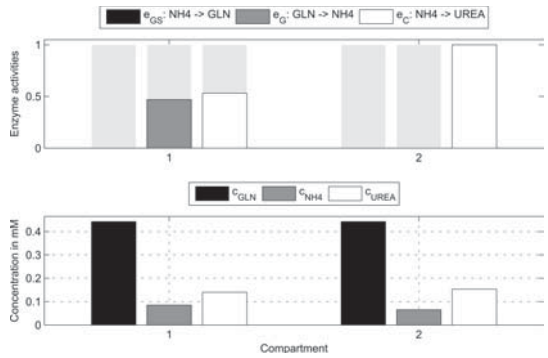


*Figure 3: Optimization results for the two-compartment model and for urea maximization only (Eq. (13)) with the obtained enzyme activity distribution (upper part) and the corresponding metabolite concentrations (lower part).*

Based on this, it may be concluded that the combination of both objective functions (Eqs. (11), (13)) would better reflect the actual physiology of the liver. This combined function can be interpreted to describe ammonia detoxification via ureogenesis, which is a major physiological task of the liver besides the control/regulation of glutamine concentration in the bloodstream.

Additionally considering glutamine control, the idea of a combined objective function can be taken a step further. The aim of glutamine control by the liver can be considered to be maintenance, i.e. to keep glutamine levels constant. This can be measured by the input/output concentrations. Here, also data from [3] was used: $c_{GLN,Input}$*=0.5mM,* $c_{GLN,Output}$*~ 0.45mM.*

With the above considerations, the extended objective function (quantifying ammonia minimization, urea maximization and glutamine maintenance) can be written as

$$\min_{e} \; \alpha_1 \cdot c_{NH4,2} - \alpha_2 \cdot c_{UREA,2} + \alpha_3 \cdot (c_{GLN,Input} - c_{GLN,2})^2 \tag{14}$$

with the weighting factors $[\alpha_1]=1/mM$, $[\alpha_2]=1/mM$ and $[\alpha_3]=1/mM^2$.

The quantitative impact of different weighting factors on the problem solution remains to be investigated in a further study.

The optimal solution obtained using Eq. (14) as objective function is shown in Fig. 4. With this solution, the original model structure is well reproduced (cf. Fig. 1).

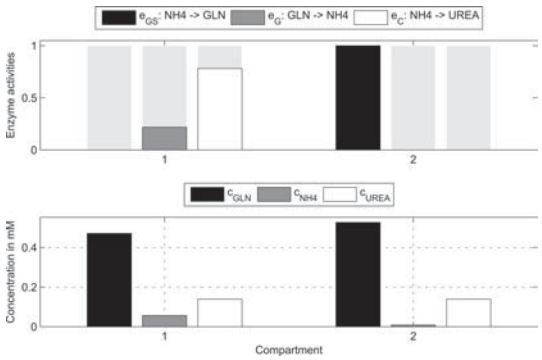


*Figure 4: Optimization results for the two-compartment model and for ammonia minimization, urea maximization and glutamine maintenance (Eq. (14)) with the obtained enzyme activity distribution (upper part) and the corresponding metabolite concentrations (lower part). The original model structure is well reproduced (cf. Fig. 1).*

In the pp zone, glutaminase and carbamoyl phosphate synthetase are active. Here, the latter is higher activated than glutaminase in order to detoxify ammonia to urea both from the input stream and the glutaminase-catalysed formation. In the pc zone, only glutamine synthetase is active and only glutamine formation takes place in order to almost completely remove ammonia. The control/regulation of glutamine is realized simultaneously.

### 4.2. MBO-based prediction for the sixteen-compartment model

In this subsection, the MBO is applied to the extended sixteen-compartment model (see Subsection 3.2.) where every compartment represents a single hepatocyte from the pp to the pc site in an average rat liver.

The obtained MBO results from Section 4.1. indicate that the objective function formulated by Eq. (14) is in line with the physiological 'objectives' of the liver. The optimization problem for the sixteen-compartment model therefore consists of the objective function

$$\min_{e} \; \alpha_1 \cdot c_{NH4,16} - \alpha_2 \cdot c_{UREA,16} + \alpha_3 \cdot (c_{GLN,Input} - c_{GLN,16})^2 \quad (15)$$

and the constraints of Eqs. (7)-(10) with *n=16*. The optimization results are shown in Fig. 5.

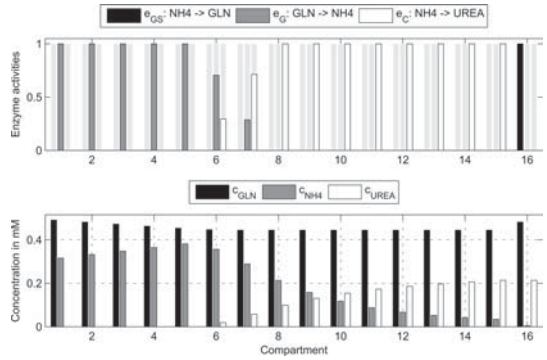


*Figure 5: Optimization results for the sixteen-compartment model and for ammonia minimization, urea maximization and glutamine maintenance (Eq. (15)) with the obtained enzyme activity distribution (upper part) and the corresponding metabolite concentrations (lower part).*

It can be seen that the activation of glutaminase in the hepatocytes (compartments) 1 to 5 builds up the ammonia concentration for an efficient detoxification to urea by carbamoyl phosphate synthetase which takes place in the following hepatocytes up to hepatocyte 15. All these cells together make up for a large 'pp' zone. Ammonia is then again almost completely removed in the pc zone that here however consists of only one, hepatocyte 16. The maintenance of glutamine is also again realized simultaneously (cf. glutamine concentrations in hepatocytes 1 and 16).

The optimal enzyme activity distribution obtained with respect to the pc zone resembles the small pc zone found *in vivo* [5] very well. There is a full and exclusive activation of glutamine synthetase only in hepatocyte 16.

The MBO results obtained using the sixteen-compartment model again support the zonation structure but additionally suggest that a further subdivision of the pp zone is apparent, since there is a more diverse enzyme activity distribution obtained for this zone (cf. Fig. 5, hepatocytes 1 to 15). Thus, according to the obtained distribution of the enzyme activities along the 16 hepatocytes, the liver lobule acinus may be divided into 4 major zones.

In the first zone (hepatocytes 1 to 5), only ammonia formation takes place. This result nicely fits to earlier unexplained experimental findings concerning the zonal expression of glutaminase [6].

In the second zone (hepatocytes 6 and 7), ammonia formation and ureogenesis take place in parallel.

In the third zone (hepatocytes 8 to 15), only ureogenesis takes place. This enzyme pattern may explain recent experimental findings suggesting glutaminase-independent ureogenesis in a downstream 'pp' region [7].

In the fourth or pc zone (hepatocyte 16), only glutamine formation needs to be performed in order to meet all physiological requirements reflected by the extended objective function Eq. (15).

## 5. CONCLUSIONS

A new MBO approach was applied in this study to investigate physiological strategies of the liver based on an established metabolic model in order to better explain the zonation of liver nitrogen metabolism.

This approach clearly demonstrated its superior predictive potential over former sole modelling approaches [1, 2].

Moreover, the fact that this approach provided plausible explanations for two unexpected and as yet unexplained experimental findings [6, 7] emphasizes that optimization and its principles may not only serve as tools extending modelling, but may also play a prominent role in liver physiology itself.

Furthermore, it can be concluded from this study that the MBO approach applied provides a promising tool for identifying novel model structures of liver zonation based on the formulation of biologically meaningful constraints and objective functions related to liver physiology.

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